

Evidence for regular ongoing introductions of mosquito disease vectors into the Galápagos Islands

Arnaud Bataille^{1,2,3,*}, Andrew A. Cunningham^{2,4}, Virna Cedeño^{4,5,6}, Marilyn Cruz⁴, Gillian Eastwood^{1,2}, Dina M. Fonseca⁷, Charlotte E. Causton⁸, Ronal Azuero⁸, Jose Loayza⁸, Jose D. Cruz Martinez⁸ and Simon J. Goodman^{1,4,*}

¹Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, UK

²Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK

³NERC Molecular Genetics Facility (Sheffield), Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

⁴Galápagos Genetics, Epidemiology and Pathology Laboratory, Galápagos National Park, Universidad de Guayaquil, Puerto Ayora, Galápagos Islands, Ecuador

⁵Concepto Azul, Cdl. Vernaza Norte Mz 10 Villa 34, PO Box 09-02-142A, Guayaquil, Ecuador

⁶Biotechnology Program, Universidad de Guayaquil, Guayaquil, Ecuador

⁷Center for Vector Biology, Rutgers University, New Brunswick, NJ 08901, USA

⁸Charles Darwin Research Station, Puerto Ayora, Galápagos Islands, Ecuador

Wildlife on isolated oceanic islands is highly susceptible to the introduction of pathogens. The recent establishment in the Galápagos Islands of the mosquito *Culex quinquefasciatus*, a vector for diseases such as avian malaria and West Nile fever, is considered a serious risk factor for the archipelago's endemic fauna. Here we present evidence from the monitoring of aeroplanes and genetic analysis that *C. quinquefasciatus* is regularly introduced via aircraft into the Galápagos Archipelago. Genetic population structure and admixture analysis demonstrates that these mosquitoes breed with, and integrate successfully into, already-established populations of *C. quinquefasciatus* in the Galápagos, and that there is ongoing movement of mosquitoes between islands. Tourist cruise boats and inter-island boat services are the most likely mechanism for transporting *Culex* mosquitoes between islands. Such anthropogenic mosquito movements increase the risk of the introduction of mosquito-borne diseases novel to Galápagos and their subsequent widespread dissemination across the archipelago. Failure to implement and maintain measures to prevent the human-assisted transport of mosquitoes to and among the islands could have catastrophic consequences for the endemic wildlife of Galápagos.

Keywords: Galápagos; *Culex quinquefasciatus*; emerging infectious disease; West Nile virus; population genetics; aircraft monitoring

1. INTRODUCTION

Human-aided transport and human-induced environmental changes have dramatically increased the capacity of disease vectors, such as mosquitoes, to reach, establish and spread in previously inaccessible areas, triggering emergence and epidemics of vector-borne diseases across the globe (Lounibos 2002). Disease vector introductions in recent history are closely linked with the development of global transportation links such as the Worldwide Airline Network (Tatem *et al.* 2006). Recent outbreaks of West Nile fever in the USA (Kramer *et al.* 2008) and of Chikungunya disease throughout the Indian Ocean region (Chevillon *et al.* 2008) are striking

examples of the impacts of globalization on human and animal health. Recently, emerging infectious diseases that have wildlife host reservoirs have been highlighted as threats to human health, the global economy and biodiversity (Daszak *et al.* 2000; Jones *et al.* 2008). Wildlife endemic to isolated oceanic islands is particularly susceptible to the introduction of infectious diseases (Matson 2006). For example, the co-introduction of the southern house mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) and avianpoxvirus in Hawaii, followed by a later introduction of avian malaria, precipitated dramatic declines and extinctions among Hawaiian endemic birds (Warner 1968; Van Riper *et al.* 1986, 2002). In contrast to Hawaii, Galápagos remains one of the most pristine archipelagos on Earth, with much of its endemic fauna intact (Tye *et al.* 2002; Watkins & Cruz 2007). The introduction of new pathogens and disease vectors could have devastating effects on the Galápagos biodiversity (Wikelski *et al.* 2004; Kilpatrick *et al.* 2006), and because of this, the establishment of *C. quinquefasciatus*

* Authors and address for correspondence: Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, L.C. Miall Building, Clarendon Way, Leeds LS2 9JT, UK (bgyaba@leeds.ac.uk, s.j.goodman@leeds.ac.uk).

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in Galápagos in the mid-1980s is considered a serious threat to its endemic fauna (Whiteman *et al.* 2005; Causton *et al.* 2006).

Culex quinquefasciatus is a member of the *Culex (pipiens) pipiens* complex found in tropical and sub-tropical regions and is an important vector of wildlife diseases such as avian malaria, avianpox (Van Riper *et al.* 1986, 2002), filariasis (Labarthe & Guerrero 2005) and West Nile fever (Sardelis *et al.* 2001). Kilpatrick *et al.* (2006) estimated that West Nile virus (WNV) would most likely reach Galápagos by means of the anthropogenic introduction of mosquitoes from mainland South America. Further introduction of infectious *C. quinquefasciatus* mosquitoes was considered to be a particular risk for WNV introduction, with the risk from aeroplane transport of infectious mosquitoes to the islands calculated as being two orders of magnitude greater than the next most likely route of introduction by migratory birds (Kilpatrick *et al.* 2006). Once introduced, the native Galápagos mosquito *Aedes taeniorhynchus* has the potential to spread WNV (or other mosquito-borne pathogens) throughout the archipelago to a wide range of endemic vertebrate taxa (Bataille *et al.* 2009).

Given these risks, it is imperative to determine if there are ongoing introductions of *C. quinquefasciatus* and, if so, to assess how effectively newly introduced mosquitoes incorporate into established populations (as this can be taken as a proxy of survival for immigrants), in order to determine ongoing risks of mosquito-borne pathogen introductions to Galápagos. Also, the identification of introduction pathways is essential for the implementation of appropriate measures to mitigate the risk of mosquito-borne disease introductions. Recent reports indicate that aeroplanes (commercial and freight) and cargo boats from mainland Ecuador represent the main routes for insect introductions to Galápagos (Causton *et al.* 2006; Kilpatrick *et al.* 2006). Once introduced to an island, insects may invade the rest of the archipelago through natural dispersal or via human-aided transport such as aeroplanes and boats. It has been shown, for example, that lights on tourist boats attract flying insects, including *C. quinquefasciatus* (Roque Albelo *et al.* 2006), increasing the likelihood of spread of insects and vector-borne diseases among the islands.

In this paper we integrate the results of an aeroplane-monitoring programme in Galápagos with a population genetics study of *C. quinquefasciatus*. The former directly assesses the rates of transport of mosquitoes to Galápagos by air from mainland Ecuador, and the latter determines the population structure of *C. quinquefasciatus*, the pathways and frequency of introduction from the mainland to the archipelago and its spread among the islands.

2. MATERIAL AND METHODS

(a) Aeroplane monitoring for mosquito introductions

Monitoring was conducted twice a month between October 2006 and September 2007 in Baltra airport and from October 2006 until March 2007 in San Cristobal airport, these being the only two airports in Galápagos that regularly receive flights from mainland Ecuador. On each monitoring occasion, the luggage compartments and the cabin areas of two to five aeroplanes were visited within an hour of arrival

from mainland Ecuador, after the passengers had left the aeroplane, and once approximately half the cargo had been removed to allow access to the hold. Any live or dead invertebrates found were collected with hand-held aspirators and butterfly nets, and transported to the Charles Darwin Research Station for storage and identification. Mosquitoes were then sent to the Galápagos National Park's Genetics, Epidemiology and Pathology Laboratory (GGEPL) for further study.

(b) Mosquito sample collection, DNA extraction and microsatellite genotyping

For the population genetics study, adult *C. quinquefasciatus* mosquitoes were collected across the five inhabited Galápagos Islands (Baltra, Floreana, Isabela, San Cristobal, Santa Cruz; 302 specimens) and from mainland Ecuador (36 specimens) (figure 1) using miniature ultraviolet light traps or miniature incandescent light traps with a photo-switch-controlled CO₂ release system (John W. Hock Company, Gainesville, FL). Light trap contents were examined either at GGEPL or at a laboratory on the mainland (Concepto Azul, Guayaquil, Ecuador). *Culex* mosquitoes were identified using morphological features, separated from other insects and collected and stored at -20°C. Additional *C. quinquefasciatus* specimens were collected as larvae from oviposition traps following the method described by Whiteman *et al.* (2005), reared to adulthood and then stored at -20°C. Six *Culex* specimens collected from the aeroplane monitoring performed in Baltra airport were also included in the study.

The abdomen was removed from each female specimen before DNA extraction and the whole body was used for male specimens. DNA was extracted using a salting-out extraction method with ammonium acetate (Nicholls *et al.* 2000). We identified the *Culex* specimens collected as *C. quinquefasciatus* by using a polymerase chain reaction (PCR)-based method (Smith & Fonseca 2004). We genotyped each mosquito individually using 11 microsatellites originally isolated from *C. quinquefasciatus* or *C. pipiens* (Fonseca *et al.* 1998; Keyghobadi *et al.* 2004; Smith *et al.* 2005) in two fluorescently labelled multiplexes (table A1 in the electronic supplementary material). Multiplex PCR was performed in a 2 µl volume containing approximately 10 ng of desiccated DNA, 0.2–0.3 µM of each primer and 1 µl of QIAGEN four-dye system Multiplex PCR Master Mix (Kenta *et al.* 2008). The PCR program used was 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 54°C for 90 s, 72°C for 60 s, and finally 60°C for 30 min. Allele sizes were assigned using an ABI3730 DNA Analyser and GENEMAPPER v. 2.0 software (Applied Biosystems).

(c) Microsatellite data analysis

For all analyses we initially grouped mosquitoes collected from different locations within each island together, and considered each grouping as a single-island population. Samples from mainland Ecuador were pooled in three groups according to their geographical origin (figure 1): a northern population (MEN), a population of the region of Guayaquil (MEG) and a southern population (MES). The six mosquitoes collected from aeroplanes in Baltra airport were considered to be of unknown origin, and were used only in individual assignment tests and tests for the detection of migrants (see following text).

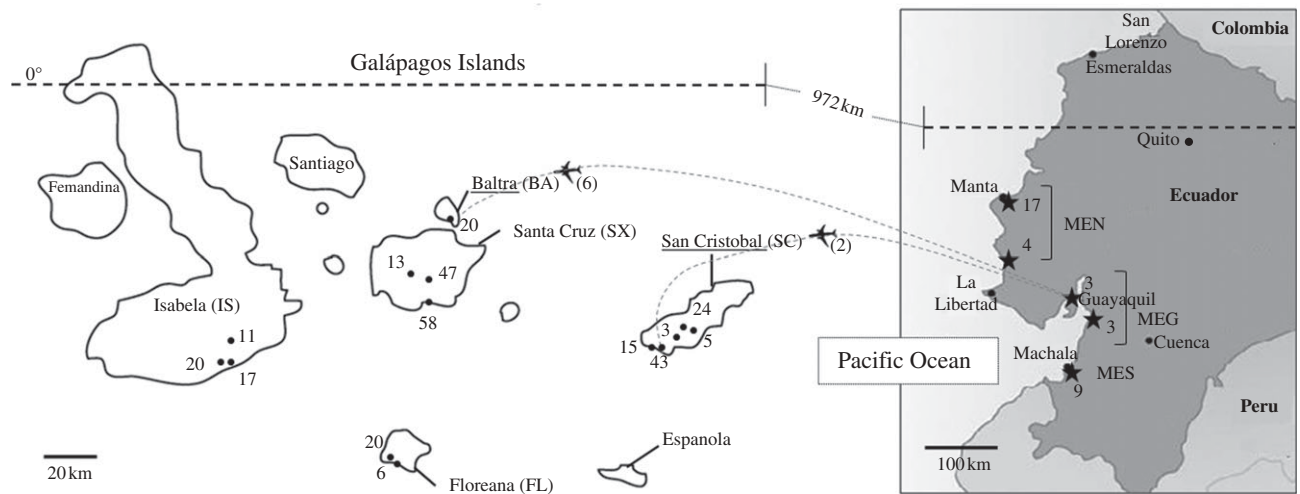


Figure 1. Mosquito sampling sites in the Galápagos Islands (dots) and in mainland Ecuador (stars). The numbers beside the sampling sites correspond to the number of *Culex quinquefasciatus* mosquitoes collected. The aeroplane images indicate flight paths to Galápagos from mainland Ecuador. The numbers in parentheses beside aeroplane images indicate the number of *C. quinquefasciatus* mosquitoes caught in aeroplanes arriving on those flight paths. MEN, mainland Ecuador North; MEG, mainland Ecuador Guayaquil; MES, mainland Ecuador South.

Heterozygosity values and estimates of the frequency of null alleles were calculated using the program CERVUS v. 3.0 (Kalinowski *et al.* 2007). Conformity to Hardy–Weinberg equilibrium and linkage disequilibrium were tested with GENEPOP 4.0 (Raymond & Rousset 1995). Genetic differentiation between populations was quantified by calculating pairwise F_{ST} (Weir & Cockerham 1984) in FSTAT v. 2.9.3 (Goudet 1995). Permutation tests with 10 000 randomizations were used to determine if the observed values were significantly different from zero, and the p -value adjusted using a sequential Bonferroni correction (Rice 1989). We also examined the population structure in our dataset with the Bayesian individual clustering method implemented in the program STRUCTURE v. 2.2 (Pritchard *et al.* 2000). We used the admixture model and tested for K potential genetic clusters contributing to the sample, with values of K ranging from 1 to 14. The program was run five times for each K for 300 000 generations with 100 000 burn-in steps (after checking for convergence of the Markov chain Monte Carlo chains), and the most likely K was identified according to the log-likelihood of the data ($[\ln P(D)]$ values), by estimation of $P(K|X)$. In addition, we performed a principal component analysis (PCA) on the gene frequency data using the program PCA-GEN v. 1.2 (<http://www2.unil.ch/popgen/softwares/pcagen.htm>) with 10 000 randomizations to test if total inertia as well as inertia for individual axes were significantly different from zero.

The program STRUCTURE v. 2.2 was used to detect migrants in populations by supplying prior information on the origin of each individual genotyped. Here we specified the individuals as coming from one of the five Galápagos islands sampled or from one of the three mainland populations (total of eight populations), and the program was run as described above for $K=8$. The six mosquitoes caught in aeroplanes were described as originating from Baltra to test if the program could identify them as migrants from mainland Ecuador. We estimated, in terms of admixture proportions, the contribution of introductions from the mainland population to the genetic diversity of each island population with LEADMIX (Wang 2003). The effective

number of migrants between each population ($4Nm$, where N is the effective population size and m the migration rate) was calculated with a Bayesian approach (Beerli 2006) implemented in the program MIGRATE v. 3.0 (Beerli 2008). The program was run initially with default setting for 10 000 000 generations with 2 000 000 generations burn-in. The outputs from the initial run were then used to parameterize three further runs of the same length, and congruence of the results verified.

3. RESULTS

(a) *Aeroplane monitoring*

Ninety-three aeroplanes were assessed for the presence of dead or live invertebrates at Baltra airport over a period of 12 months (October 2006 to September 2007) and 33 aeroplanes were assessed at San Cristobal airport over a 6 month period (October 2006 to March 2007). We collected 105 invertebrates, 74 of which were alive (table A2 in the electronic supplementary material). Eleven of the live invertebrates collected were mosquitoes; no dead mosquitoes were found. Three specimens of *A. taeniorhynchus* and six specimens of *C. quinquefasciatus* mosquitoes were caught in aeroplanes landing at Baltra airport, and two *C. quinquefasciatus* were caught in aeroplanes landing at San Cristobal airport (these two specimens were not available for further genetic analysis). All *A. taeniorhynchus* and five *C. quinquefasciatus* mosquitoes were caught during the mainland wet season (January to March 2007; table A2 in the electronic supplementary material).

(b) *Microsatellite variation, linkage disequilibrium and Hardy–Weinberg equilibrium*

The number of alleles per locus ranged from 4 to 15 and the expected heterozygosity ranged from 0.30 to 0.75 (table A1 in the electronic supplementary material). Tests for linkage disequilibrium between all pairs of loci across populations found no significant disequilibrium after a sequential Bonferroni correction. Exact tests

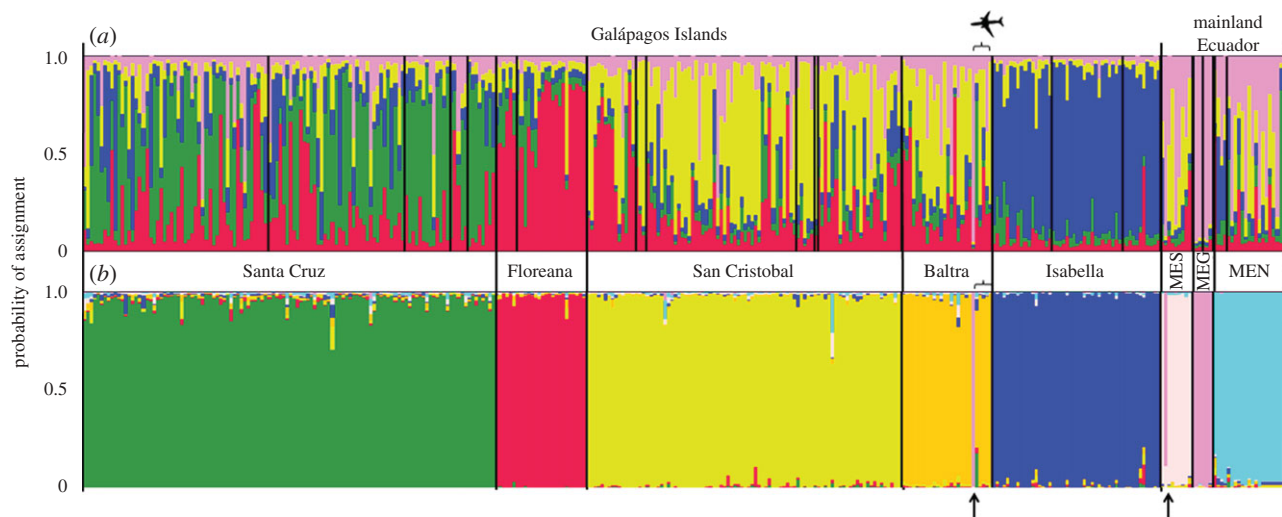


Figure 2. Clustering of *Culex quinquefasciatus* individuals from the Galápagos Islands and mainland Ecuador based on the STRUCTURE 2.2 algorithm (Pritchard *et al.* 2000). Each of 343 individuals is represented by a vertical bar partitioned in colours segmented according to the probability of belonging to one of the K genetic clusters. (a) No prior information on population is given and K is defined as the number of genetic clusters that best fit with the data (here $K = 5$, identified by the five colours in the graph). (b) Prior information on population is given ($K = 8$, according to geographical locations) to detect potential migrants. Mosquitoes caught in aeroplanes are grouped and indicated by an aeroplane image. Potential migrants are identified by an arrow. MEN, mainland Ecuador North; MEG, mainland Ecuador Guayaquil; MES, mainland Ecuador South.

showed that locus pGT46 deviated significantly from the Hardy–Weinberg expectations in four of eight populations analysed (table A3 in the electronic supplementary material). These deviations were associated with positive F_{IS} values ranging from 0.194 to 0.417, reflecting heterozygosity deficiency, probably owing to a high frequency of null alleles in pGT46 (estimated with CERVUS to range from 0.18 to 0.24). Subsequent analyses were performed without the locus pGT46 to ensure that results would not be biased by the presence of null alleles.

(c) Population structure

Of 28 F_{ST} values for pairwise comparisons between locations, 26 were significantly different from zero (range 0.023–0.35, $p < 0.001$; table A4 in the electronic supplementary material) and all remained significant after sequential Bonferroni correction. F_{ST} values showed that there was no significant differentiation between mosquito populations sampled in Baltra and San Cristobal Islands, or between the northern and southern mainland populations. F_{ST} values between Baltra and San Cristobal Islands and the northern and southern mainland populations were small but significant (table A4 in the electronic supplementary material).

The Bayesian clustering analysis in STRUCTURE 2.2 determined $K = 5$ to have the highest probability ((for $K = 5$, $P(K|X) = 1$, for all other values of K , $P(K|X) = 0$); figure A1 and table A5 in the electronic supplementary material). Individuals of Santa Cruz, Isabela and Floreana Islands were predominantly assigned to three clusters, each specific for each island, whereas *C. quinquefasciatus* individuals from Baltra and San Cristobal Islands were clustered together and mainland individuals were grouped in a fifth cluster (figure 2a). Only two mosquitoes out of six caught in aeroplanes in Baltra airport were assigned to the mainland genetic cluster. Three of the remaining individuals were assigned to the Baltra–San Cristobal cluster, and the last one was

assigned to the Santa Cruz cluster (figure 2a). In the PCA, the first principal component accounted for 41.6 per cent of the inertia in the data and separated Santa Cruz, Isabela and Floreana Islands from Baltra and San Cristobal Islands, which clustered with the three mainland populations (figure 3). The second principal component accounted for 22.8 per cent of the inertia in the data and separated Isabela Island from Santa Cruz and Floreana Islands (figure 3).

(d) Detection of potential migrants

Analyses in STRUCTURE 2.2 incorporating prior information on the origin of each individual identified two potential migrants (figure 2b). Both individuals, one collected in an aeroplane that landed in Baltra Island and another from the southern mainland population, were identified as migrants from the Guayaquil region in mainland Ecuador (figure 2b). The other five mosquitoes caught in aeroplanes were assigned to the Baltra genetic cluster (figure 2b).

(e) Admixture proportions and migration patterns

Admixture analyses with LEADMIX showed that the Baltra and San Cristobal populations (the sites of the airports) have a mixture of alleles from both the established Galápagos populations and the mainland populations, with an input from the mainland population of 50.7 per cent (confidence intervals (CI) 28–77%) in Baltra and of 33.6 per cent (CI 11.5–56.4%) in San Cristobal. The estimated admixture between the mainland populations and Isabela and Floreana had CIs overlapping zero, and so were not significant. The mainland input in the Santa Cruz population was significantly smaller than in Baltra and San Cristobal populations (3.2%; CI 0.7–10.6%). We then assessed the input from Baltra and San Cristobal Islands separately into each of the three remaining islands. The admixture levels obtained had

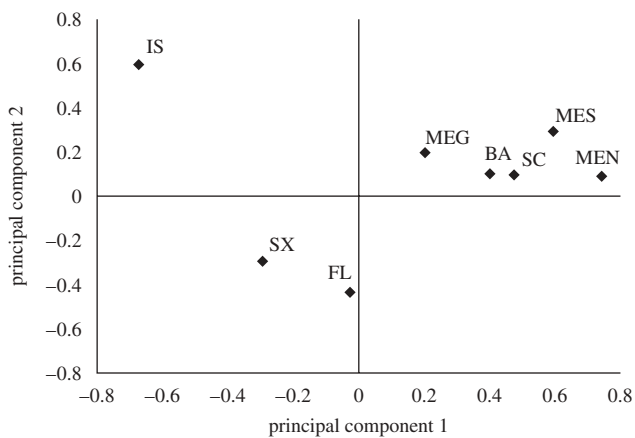


Figure 3. Plot of the Eigen-values for the two first components of the principal component analysis (PCA) performed on *Culex quinquefasciatus* populations from Galápagos Islands and mainland Ecuador. IS, Isabela; SX, Santa Cruz; SC, San Cristobal; BA, Baltra; FL, Floreana; MEN, mainland Ecuador North; MES, mainland Ecuador South; MEG, mainland Ecuador Guayaquil.

large confidence intervals often overlapping zero, making the results hard to compare. However, there is some suggestion that input from both Baltra and San Cristobal may be important in Floreana Island (43.6 and 43.7%; CI 0.4–87%) and in Isabela (24.6 and 25.7%; CI 0–73.9%). In the Santa Cruz population, some allelic input may come from San Cristobal (12.6%; CI 0–66%), but probably not from Baltra (0.01%; CI 0–12.2%).

Precise estimation of migration rates between populations using MIGRATE was problematic for our data, because the estimates had large CIs (table A6 in the electronic supplementary material). Nevertheless, results indicated that the highest level of migration was from mainland Ecuador towards Baltra Island ($4Nm = 5.23$; CI 95%, 3–9.9) and San Cristobal Island (5.22; CI 1.6–9.95; table A6 in the electronic supplementary material). Our results also showed that there is some gene flow from Santa Cruz Island population towards Isabela (0.92; CI 0.17–1.68), San Cristobal (1.22; CI 0.6–2.62) and Baltra Islands (1.66; CI 0.46–4.62). Some gene flow was also detected from Isabela towards San Cristobal Island (2.33; CI 0.1–4.35) and from Baltra towards Floreana (2.24; CI 0.33–5.27). The results also indicated some gene flow between Floreana Island and mainland Ecuador (2.17; CI 0.55–4.86) and high numbers of migrants between Baltra and San Cristobal (5.88; CI 2.15–10; table A6 in the electronic supplementary material); the latter is probably owing to the influence of the continental mosquito populations on the mosquito populations of Baltra and San Cristobal Islands.

4. DISCUSSION

Many invertebrate specimens from a wide diversity of orders were collected during the aeroplane monitoring, indicating that the introduction of invasive species by aeroplane represents a continuing and serious threat to the conservation of the endemic biodiversity of Galápagos, especially during the rainy season (Causton *et al.* 2006). The presence of *C. quinquefasciatus* specimens in

aeroplanes combined with our genetic data suggests that this species has been introduced frequently to the Galápagos Archipelago by air transportation since it was first identified in Galápagos (on Santa Cruz Island) in 1985 (Whiteman *et al.* 2005). The genetic similarity between Baltra, San Cristobal and mainland *C. quinquefasciatus* populations (figures 2 and 3) suggests that arrival rates, survival and subsequent breeding with established island populations are high, and the Galápagos population is not therefore the result of a single colonization event.

Most aircraft landing in Galápagos transit through the international airport of Guayaquil (Cruz Martinez & Causton 2007), Ecuador's largest city, which is situated on the edge of the Guayas river delta. Within the airport perimeter are a large number of drainage ditches, potentially providing a good breeding habitat for *C. quinquefasciatus*. Therefore, it is most likely that mosquitoes introduced to Galápagos originate from Guayaquil airport, rather than from Quito, the origin for most Galápagos passenger flights, but which is at a high altitude (approx. 2800 m) and has much lower mosquito populations. Our results indicate that the Baltra and San Cristobal populations might be more closely related to the southern and northern Ecuadorian mainland populations than to the Guayaquil population. However, few samples were available from the Guayaquil region and southern Ecuador (6 and 9 individuals, respectively; figure 1), which might not be sufficient to give a true representation of the genotype frequencies in these areas. Alternatively, some mosquitoes might have survived on aircraft that had travelled to Guayaquil from other cities in the Ecuadorian domestic airline network, and that were subsequently routed to Galápagos via Guayaquil. Another option is that mosquitoes might have been introduced directly from elsewhere in Ecuador prior to the current restrictions on the routing of Galápagos-bound flights via Guayaquil.

Cargo boats transporting food, agricultural supplies and other goods to Galápagos have also been recognized as an important route for the introduction of invasive insect species (Causton *et al.* 2006). Santa Cruz, San Cristobal and Baltra islands are the locations for the main sea ports of Galápagos. At Santa Cruz, cargo boats unload in Puerto Ayora on the southern side of the island, approximately 45 km from Baltra airport, whereas on San Cristobal both the port and the airport are situated in the town of Puerto Baquerizo Moreno. Baltra has a small deep-water port next to the airport. Admixture analysis showed that the direct continental input into the *C. quinquefasciatus* population of Santa Cruz was small (3.2%), and the PCA and F_{ST} values indicated significant differentiation of the Santa Cruz population from the Baltra, San Cristobal and mainland populations (figure 3). This suggests that introductions of *C. quinquefasciatus* to Santa Cruz Island by boat are much less important than introductions by aeroplane (or boat) to Baltra and San Cristobal.

Our results also provide evidence for movements of mosquitoes between the islands of the archipelago. *Culex quinquefasciatus* has poor flying capacity and would not normally disperse successfully from human settlements because of the scarcity of fresh water on Galápagos. Therefore any movements of *Culex* mosquitoes among islands are most probably human-aided.

Our results support this hypothesis, as movements detected with MIGRATE seem to originate from Santa Cruz, which is the most populated island of the archipelago. The port of Santa Cruz Island, Puerto Ayora, is a local maritime transport hub for the other islands, and a stop off for most tourist cruises. Gene flow was also detected between Baltra and Floreana Islands, which are not linked by local transport. This suggests that tourist cruises, most of which start from Baltra, may play a significant role in the movement of mosquitoes between islands (Roque Albelo *et al.* 2006).

Our results emphasize that the transport of mosquitoes by aeroplane remains one of the most important risk factors for vector-borne disease introduction to Galápagos, as previously proposed (Kilpatrick *et al.* 2006). Kilpatrick *et al.* (2006) used data on mosquito transport on aircraft from other parts of the world to parameterize a risk assessment model for WNV introduction to Galápagos. Using direct observations for flights to Galápagos, we calculated that an average of 0.098 live mosquitoes arrived per aeroplane at Baltra airport and 0.061 mosquitoes per aeroplane at San Cristobal airport. When estimations were made on results from the wet season only (January to March 2007), the average number of mosquitoes per aeroplane increased to 0.19 in Baltra airport. These numbers are lower than previously estimated (Kilpatrick *et al.* 2006), but we may have missed mosquitoes escaping from aeroplanes before we were allowed to enter them. The number of commercial flights has almost doubled from the 2004 values used by Kilpatrick *et al.* (2006) (Cruz Martinez & Causton 2007). Using these updated data along with our Galápagos-specific mosquito arrival rates and following the methods of Kilpatrick *et al.* (2006), we can estimate a minimum risk of 4.33 (CI 1.09–10.89) WNV-infectious mosquito days being introduced to Galápagos per year if WNV becomes established in the mainland Ecuador mosquito population. This risk increases to 13.48 (CI 3.41–33.92) infectious mosquito days per year during the rainy season. These compare with an overall air transportation risk of 101.7 (CI 8.3–272.9) infectious mosquito days originally calculated by Kilpatrick *et al.* (2006). Although the absolute values using the updated data are lower, the CIs overlap with those of the original estimate.

Proof of regular introduction of mosquitoes by aeroplane is a great concern and the risks of introduction and spread within the archipelago scale directly with the increase in flights and in visitor numbers (Cruz Martinez & Causton 2007). Moreover, there is continuing pressure to allow flights from other Ecuadorian airports to Galápagos and to open a newly extended airport at Puerto Villamil on Isabela Island to flights from the mainland. Aeroplane disinsection, according to standard World Health Organization protocols, have been mandatory for commercial flights from mainland Ecuador to Galápagos since August 2007. This should greatly reduce the threat of live mosquito introductions, but monitoring of the success of the disinsection is required to ensure its effectiveness. Also, disinsection must be an enforced requirement for *all* aircraft (commercial passenger, freight, military or private) landing in the Galápagos Islands to minimize the risk of transporting any invasive invertebrates to the archipelago.

Disinsection protocols have recently been implemented for inter-island aeroplane services, which should help reduce risks of transport via this route. For sea routes, a quarantine and inspection system is in place to control the movement of goods between islands (Causton *et al.* 2006), but presently it does not have the means to effectively prevent the transport of stowaway invertebrates on tourist or local boats. Ideally, fumigation or disinsection protocols for tourist and local boats should be implemented, with strict measures especially taken for boats departing from Baltra, San Cristobal and Santa Cruz. Roque Albelo *et al.* (2006) recommended measures such as using insect traps and permitting only yellow lights to be used at night by boats to limit the transportation of insects. Ideally, these would be made standard procedure in Galápagos.

This study is the first, to our knowledge, to present genetic evidence showing the role of the Worldwide Airline transportation Network (Tatem *et al.* 2006) in the invasion of isolated oceanic islands by disease-carrying mosquitoes. Oceanic islands such as the Galápagos are losing this isolation and it might represent one of the most important threats to the endemic biodiversity and the economies of these environments. With steadily growing transport links between the Galápagos Islands and the continent, strict mitigation measures must be implemented and maintained, otherwise invasive species and pathogens, such as WNV, are certain to reach and spread among the Galápagos Islands, with a potentially devastating effect on the endemic wildlife.

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